Journal of Medical Virology 52:286–288 (1997)

Lack of Susceptibility of the Cottontop Tamarin to Hepatitis C Infection

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The only species apart from man that is known to be susceptible to HCV infection is the chimpanzee but the availability of this primate for research is strictly limited. In an attempt to find an alternative and more practical model for HCV studies three cottontop tamarins were inoculated intravenously with HCV-containing serum from patients with chronic HCV infection. The tamarins were monitored regularly for biochemical indications of hepatic inflammation and serum samples were assayed at weekly intervals for the presence of HCV-RNA and HCV antibodies. HCV-RNA was detectable at 10 minutes postinoculation in all three animals but not at any later time point over a 6 month period. No evidence of an active humoral immune response to the inoculated HCV was obtained although passively transferred anti-HCV was detectable in one animal until 1 week postinoculation. Biochemical findings did not indicate hepatic inflammation and liver histology remained normal. It is concluded on the basis of these negative findings that the cottontop tamarin is not susceptible to HCV infection. ***J. Med. Virol. 52: 286–288, 1997.***

KEY WORDS: hepatitis C virus; cottontop tamarin; PCR; animal model; inoculation

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of parenterally acquired hepatitis and represents a significant health problem in many parts of the world. Although the acute disease is generally mild at least half of the individuals infected develop chronic hepatitis and approximately 20% of these eventually progress to cirrhosis with its associated risk of hepatocellular carcinoma [Di Bisceglie et al., 1991; Tsukuma et al., 1993].

Progress in HCV research has been hampered by the lack of a practical animal model. The availability of such a model would provide a system for the study of pathological mechanisms and greatly facilitate the development and evaluation of vaccines and antiviral drugs for the prevention and treatment of HCV infection. At present the only species apart from man that is known to be susceptible to HCV is the chimpanzee [Farci et al., 1993]. Although the chimpanzee is a useful model in many respects it is now virtually unobtainable for research purposes. Studies with other primates, notably macaques, cynomolgus monkeys and two tamarin species, *Saguinus mystax* and *Saguinus labiatus,* have suggested that they may also be susceptible to non-A, non-B agents [Tabor, 1989]. However, these studies were undertaken before the non-A, non-B hepatitis virus group had been subdivided into identifiable agents and before specific diagnostic tests for HCV were available.

We therefore decided to investigate the possibility that the cottontop tamarin, *Saguinus oedipus oedipus,* might be a suitable model for HCV infection. The cottontop tamarin was selected because it has been used extensively for many years in Epstein-Barr virus research and because it has been shown to be a practical animal model for viral pathogenesis studies and vaccine trials [Morgan, 1996; Finerty et al., 1992].

MATERIALS AND METHODS

Animals and Veterinary Procedures

One male and three female cottontop tamarins, bred in the colony at the University of Bristol, were taken from the main colony and housed under Category 2 containment conditions in a negative pressure isolator. The animals were aged between 3 and 5 years. None had been inoculated previously with any hepatitis virus, blood, or plasma derived material. All regulated procedures were carried out under sedation (ketamine, Parke Davis, Pontypool, Gwent, UK; medetomidine, Pfizer, Sandwich, Kent, UK) or general anaesthesia (alphlaxone, Mallinckroot, Harefield, Midox, UK).

Inocula

Three high titre serum samples from HCV-infected patients were selected for inoculation. All had been

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Accepted 12 February 1997

separated within a few hours of venesection, snap frozen in liquid nitrogen, and stored at –70°C. One was genotype 4 (Simmonds classification), another genotype 1, and the third was untypeable. The titre of the genotype 4 sample was 3×10^6 HCV genomes/ml, both of the other sera had HCV-RNA titres of 7×10^7. A serum sample from a healthy non-infected individual was used as a control inoculum. All the sera employed were negative for markers of human immunodeficiency virus (HIV1&2), human T-cell leukaemia virus (HTLV1&2), and hepatitis B virus infection.

Virological, Biochemical, and Histological Monitoring

HCV-RNA concentrations in the inocula and in tamarin serum samples were determined by a quantitative chemiluminescence-based polymerase chain reaction (PCR) assay [Whitby and Garson, 1995] using primers derived from the highly conserved 5′ noncoding region. The assay cut-off was approximately 10^3 HCV genomes/ml. Spiking experiments with known amounts of HCV-RNA were carried out to confirm that cottontop tamarin serum was a suitable analyte and that it did not inhibit either reverse transcription or PCR. Anti-HCV antibodies were sought using a commercial ELISA (Ortho Diagnostic Systems, Raritan, NJ) modified by using an alternative peroxidase conjugate (Sigma, St. Louis, MO; #A-8667 at a dilution of 1:2000) to be able to detect both human and tamarin IgG. The inocula and the tamarin serum samples for PCR and antibody analysis were transported between London and Bristol frozen on dry ice.

Biochemical assessment of liver function was undertaken by monitoring levels of bilirubin, gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, globulin, and total protein. The above assays were carried out on tamarin sera in the Department of Chemical Pathology, Bristol Royal Infirmary using standard automated methods. Liver tissue was examined postmortem by light microscopy using standard histological staining techniques.

RESULTS

Four animals were inoculated intravenously, one received normal human serum and each of the others received a different HCV-containing serum. The volume of serum administered was 0.5 ml in each case. Prior to inoculation serum samples from all four animals were negative for both HCV-RNA and anti-HCV antibodies. Following inoculation, venous blood samples were obtained at 10 minutes (from the contralateral limb), 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 11 weeks, 13 weeks, and monthly thereafter until 6 months postinoculation. One hundred μl aliquots of serum were assayed for HCV-RNA at each time point.

In each of the three HCV-inoculated animals HCV-RNA was detected in the samples taken at 10 minutes postinoculation but not in any of the later samples. The titre of HCV-RNA detected at 10 minutes was approximately fifty-fold lower than that of the inoculum, a reduction consistent with the dilution effect resulting from mixing of the inoculum with the blood volume. HCV antibodies were detected in the 10 minute and the 1 week samples in one of the HCV-inoculated animals only. At 10 minutes the ELISA reading was 2.09 OD units and at 1 week 0.69 OD units. The anti-HCV ELISA cut-off was 0.64 OD units.

None of the enzyme, bilirubin, or protein assays on sera from the HCV-inoculated animals or control animal showed any significant deviation from the values obtained preinoculation and there was no evidence of weight loss (range 440–560 g) throughout the experiment. Liver histology was normal in all animals and HCV-RNA was not detected in liver tissue by PCR.

One of the HCV-inoculated animals died suddenly at 4 weeks postinoculation but the death was not apparently HCV-related. Serum and liver samples taken at the time of death were negative by PCR for HCV-RNA and the liver histology was normal. Postmortem examination including histological assessment of the major organs did not reveal the cause of death.

DISCUSSION

The presence of HCV-RNA in the circulation of all three animals at 10 minutes postinoculation confirms that the intravenous inoculations were technically successful. No serological evidence of an antibody response to the inoculated HCV was obtained. The presence of anti-HCV in the 10 minute sample, and of a much lower level in the 1 week sample, from one of the HCV-inoculated animals is consistent with the detection of passively transferred anti-HCV from the inoculum rather than of an active immune response.

Since the volume of high titre HCV serum injected would have been more than adequate to infect a susceptible host (infection following needle-stick accidents in man are well documented) [Okamoto et al., 1992], we conclude on the basis of these negative findings that the cottontop tamarin is not susceptible to HCV infection. This conclusion is in accord with the observations of Abe et al. [1993] who demonstrated the inability of HCV to infect a number of other non-human primates including Cynomolgus monkeys, Rhesus monkeys, Green monkeys, Japanese monkeys, and Doguera baboons. Our inability to infect the cottontop tamarin with HCV in vivo is also consistent with our inability to infect cottontop tamarin derived B-lymphocytic cell lines with HCV in vitro [unpublished observations]. It appears therefore that the host range of HCV is extremely narrow, possibly being limited exclusively to chimpanzees and man.

ACKNOWLEDGMENTS

This work was generously supported by a grant from the Wellcome Trust.

The study was undertaken in accordance with the ethical rules for experimental animal research of the Institution.

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